Outbreak of campylobacter food-poisoning in Northern Ireland

J. E. Moore[1]*, T. Stanley*[1]*, R. Smithson*[2]*, H. O'Malley*[1] *and P. G. Murphy*[1]

[1]Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast BT9 7AD, [2]Western Health & Social Services Board, Londonderry, Northern Ireland, UK

*Tel: + 44 28 9026 3554 Fax: + 44 28 9032 1084 E-mail: jemoore@niphl.dnet.co.uk

Accepted 14 April 2000

Enteropathogenic *Campylobacter jejuni* and *C. coli* presently comprise the most common cause of acute bacterial gastroenteritis in the developed world, as well as being important gastrointestinal pathogens in developing and underdeveloped countries. The majority of human cases (99%) present as sporadic in nature, unlike other gastrointestinal infections, such as those caused by *Salmonella* Typhimurium, which are generally more related to large institutional outbreaks. However, it is unclear whether or not the epidemiologies of human campylobacter infections are related, due to poorly developed and evaluated subspecies typing schemes for this organism.

We report on an outbreak of food-poisoning, caused by *C. jejuni* among Environmental Health Officers (EHOs) attending a lunch in the west of Northern Ireland, over a 6-day period (Figure 1). Fecal specimens were received from 21 EHOs attending the lunch and were examined conventionally at the Northern Ireland Public Health Laboratory (NIPHL), Belfast City Hospital, for the presence of *Campylobacter* spp., by direct plating onto Preston's selective agar [1]. Of these, nine were positive. In order to enhance the growth of potentially small numbers of *Campylobacter* spp., all culture-negative fecal specimens were selectively enriched in Preston's broth culture; of these, one further specimen became positive. All isolates were confirmed by standard phenotypic laboratory methods as *C. jejuni* and gave the following antibiogram by disk susceptibility testing: resistant to penicillin (2 iu), cephalexin (30 μg) and trimethoprim (2.5 μg); sensitive to chloramphenicol (10 μg), erythromycin (5 μg), gentamicin (10 μg), nalidixic acid (30 μg), tetracycline (10 μg) and ciprofloxacin (1 μg), with the exception of one isolate which was resistant to ciprofloxacin.

Molecular epidemiologic studies were initiated in order to examine the genetic relatedness among the 10 fecal *Campylobacter* isolates obtained. Polymerase chain reaction–single-stranded conformational polymorphism (PCR-SSCP) [2] of a 1.7-kb region of the flagellin (*flaA*) gene following endonuclease restriction with *Dde*I [3], and multilocus enzyme electrophoresis (MEE) [4], examining nine cellular enzyme loci, were carried out in parallel. PCR-SSCP relies on the differential electrophoretic mobility of single-stranded DNA under denaturing conditions, as nucleotide substitutions will induce conformational changes, leading to detectable mobility shifts. Thus, differences between DNA banding patterns represent markers that are valuable in confirming outbreaks, as well as tracing organisms around the environment. The PCR amplicon (1.7 kb) was initially restricted with *Dde*I to yield small restriction fragments (< 600 bp), which were further separated by SSCP analysis. MEE detects polymorphisms in the gene loci coding for protein enzymes on a starch gel, and previous work has shown that this method is able to detect very fine differences between isolates.

Phenotyping and molecular genotyping expertise is normally not present in most primary diagnostic clinical laboratories, due to the relative complexity and high-costs associated with having such facilities available locally. Consequently, small local outbreaks due to this organism may not be detected, where a rapid and local response for the clinical microbiology laboratory is essential in order to aid with public health epidemiologic investigations and containment of contaminated foodstuffs or water. Presently, with a lack of phenotypic and genotypic schemes available locally, subspecies strain relatedness is crudely based on a number of qualitative phenotypic parameters, such as differences in colony morphology, and on limited antibiogram typing, based on a qualitative comparison of a limited number of disk diffusion antibiotic susceptibility assays.

In this study, PCR-SSCP and MEE gave a single fingerprint and electrophoretic type (ET), respectively, indicating that the isolates involved in this outbreak were genetically indistinguishable. Although the genetics of *Campylobacter* used in subtyping methods are hypervariable, the genotype seen in this outbreak, although relatively uncommon, was previously isolated from a 16-year-old male patient with gastroenteritis at Lagan Valley Hospital on 23 May 1994. In addition, the antibiogram profile of the *C. jejuni* strain observed in this teenager matched that of the outbreak clone.

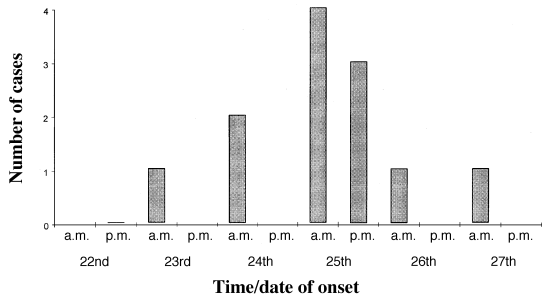

Figure 1 Epidemic curve of *Campylobacter jejuni* food-poisoning associated with a lunch in Northern Ireland in May 1997.

With regard to the epidemiologic findings relating to this outbreak, 40 people participated in the lunch, of whom 37 returned a questionnaire. The only food at the lunch which showed a statistically significant relationship with being a case was seasonal leaves and tomato salad ($P < 0.02$). Twelve definite cases were identified; of these, 11 ate the seasonal leaves and tomato salad, compared with 13 out of 25 who were not sick after eating it. Therefore, of the 24 who ate the salad, 11 became sick, giving an attack rate of 46%. Of the 12 cases, 11 reported diarrhea, 11 had abdominal cramps, eight experienced nausea, seven had fever and two experienced vomiting. The inspection of the kitchens had revealed a number of poor practices, which could have led to possible risks of contamination. These included the following:

1. Incorrect storage of foodstuffs in the walk-in chiller.
2. Roast chickens were stored under fresh meat, and roast quails were stored at the same level as raw meat. There was a lot of food left uncovered, with dishes stacked on top each other.
3. Possible misuse of food preparation surfaces, despite use of color-coding; only one red chopping board (for raw meat) was available for the two kitchens and a number of white boards were in use.
4. Close proximity of areas for the preparation of raw and cooked foods.

By the time that the relevant regulatory authorities became aware of the outbreak and the investigation started, there were no food samples left from the suspected meal and therefore there was no microbiological investigation of food relating to this outbreak.

Overall, the epidemiology of *Campylobacter* enteritis is compromised by the lack of standardized identification and typing schemes. However, improved and standardized subtyping techniques, along with isolate archives, will allow for the elucidation of important environmental sources and routes of transmission to humans.

In conclusion, this report is interesting as it describes an outbreak of *Campylobacter* enteritis, which is normally regarded as being predominantly sporadic in nature, identified as being probably due to cross-contamination in the food preparation area of the kitchen, as well as the application of a novel genotyping method (PCR-SSCP) for this organism, supported by MEE analysis.

ACKNOWLEDGMENTS

This work was funded by the Department of Health and Social Services (Northern Ireland).

The authors are grateful to the Environmental Health Officers involved in this work.

REFERENCES

1. Bolton FJ, Robertson L. A selective medium for isolating *Campylobacter jejuni/coli*. *J Clin Pathol* 1982; 35: 462–7.
2. Hayashi K. PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA. *PCR Meth Applications* 1991; 1: 34–8.
3. Nachamkin I, Bohachick K, Patton CM. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J Clin Microbiol* 1993; 31: 1531–6.
4. Aeschbacher M, Piffaretti JC. Population genetics of human and animal enteric *Campylobacter* strains. *Infect Immun* 1989; 57: 1432–7.